

# The Role of Complement in Age-Related Macular Degeneration: Heparan Sulphate, a ZIP Code for Complement Factor H?

Alex Langford-Smith<sup>a</sup> Tiarnan D.L. Keenan<sup>a–c</sup> Simon J. Clark<sup>b, c</sup> Paul N. Bishop<sup>b, c</sup>  
Anthony J. Day<sup>a</sup>

<sup>a</sup>Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, and <sup>b</sup>Centre for Hearing and Vision Research, Institute of Human Development, University of Manchester, and <sup>c</sup>Centre for Advanced Discovery and Experimental Therapeutics and Manchester Royal Eye Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK

## Key Words

Complement factor H · Age-related macular degeneration · Heparan sulphate · Tissue-specific immune recognition

## Abstract

Age-related macular degeneration (AMD) is the leading cause of blindness in developed nations and has been associated with complement dysregulation in the central retina. The Y402H polymorphism in the complement regulatory protein factor H (CFH) can confer a >5-fold increased risk of developing AMD and is present in approximately 30% of people of European descent. CFH, in conjunction with other factors, regulates complement activation in host tissues, and the Y402H polymorphism has been found to alter the protein's specificity for heparan sulphate (HS) – a complex polysaccharide found ubiquitously in mammals. HS, which is present on the cell surface and also in the extracellular matrix, exhibits huge structural diversity due to variations in the level/pattern of sulphation, where particular structures may act as 'ZIP codes' for different tissue/cellular locations. Recent work has demonstrated that CFH contains two HS-binding domains that each recognize specific HS ZIP codes, allowing differential recognition of Bruch's membrane (in the eye) or the glomerular basement membrane (in the kidney).

Importantly, the Y402H polymorphism impairs the binding of CFH to the HS in Bruch's membrane, which could result in increased complement activation and chronic local inflammation (in 402H individuals) and thereby contribute to AMD pathology.

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## Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness and visual impairment in developed countries and its prevalence is likely to increase over time, given the ageing population [1]. There is an increasing body of genetic [2–6] and biochemical [7] evidence that AMD is a disease of complement dysregulation, and therefore, that the innate immune system has a key role in the pathogenesis of the disease.

AMD is a slow, progressive, degenerative disease strongly associated with ageing that usually occurs after the age of 50 years. In populations of European ancestry, the prevalence of advanced AMD is 1.4% at 70 years of age,

A.L.-S. and T.D.L.K. contributed equally to this work.

rising to 5.6% at 80 and 20% at 90 years of age [8]. In the early stages of the disease, visual symptoms may be absent or minimal. However, in advanced cases, AMD leads to severely reduced central vision and loss of visual acuity. Clinically, AMD is subclassified into early, intermediate and late forms [9]. Early and intermediate forms are characterized by the presence of drusen, which are extracellular deposits containing proteins (including complement components) [7, 10] and lipids that accumulate between the retinal pigment epithelium (RPE) and Bruch's membrane (fig. 1a) [9, 11]. The late form is subdivided into either atrophic ('dry') or neovascular ('wet') (fig. 2). Atrophic AMD is characterized by cell death in the retina and choroid in areas where significant levels of drusen have accumulated, whereas neovascular AMD is caused by new blood vessels growing from the choroid into the retina.

### The Role of Complement in AMD: Genetics

Complement was first postulated to be involved in the pathogenesis of AMD following the discovery of complement activation products in drusen [10]. This suggestion has been strongly supported by genetic evidence, including the association of the Y402H polymorphism in complement factor H (CFH) with an increased risk of AMD [see ref. 2–4, 6, and references within]. The Y402H polymorphism, which results in a coding change of tyrosine to histidine at position 402 (position number 384 in the mature protein) [12, 13], is common in European-descended populations, with a recent meta-analysis of 26 studies estimating the prevalence of the 402H allele at 30% [4]. This meta-analysis also showed that 402H heterozygotes have a 2.3-fold and 402H homozygotes a 5.2-fold increased risk of advanced AMD [4]. Interestingly, the high prevalence of the Y402H polymorphism in people of European ances-

try fits with the hypothesis that the Y402H polymorphism acted as a survival factor by having lower binding affinity for the bubonic plague bacteria *Yersinia pestis* [14].

Other genetic variations around the CFH/CFHR-related protein (CFHR) gene cluster also modify the AMD risk including a relatively common deletion of *CFHR1* and *CFHR3*, which is protective against AMD [5]. As shown in table 1, several other genes in the complement pathway are also associated with AMD (but more weakly), including variants involving complement component 2 (*C2*), complement factor B (*CFB*), *C3*, *C9* and complement factor I (*CFI*) [2, 15].

Alongside the CFH/CFHR locus on chromosome 1, there is also a strong association with a locus at 10q31, close to the *ARMS2* and *HTRA1* genes [2]. However, it is not clear which of these genes is affected, and additional work is needed to determine the nature of their involvement in AMD pathogenesis [2]. Other weak genetic associations have been made recently based on genome-wide association studies, implicating angiogenesis, extracellular matrix and lipid metabolism in the pathology of AMD (table 1) [2, 3].

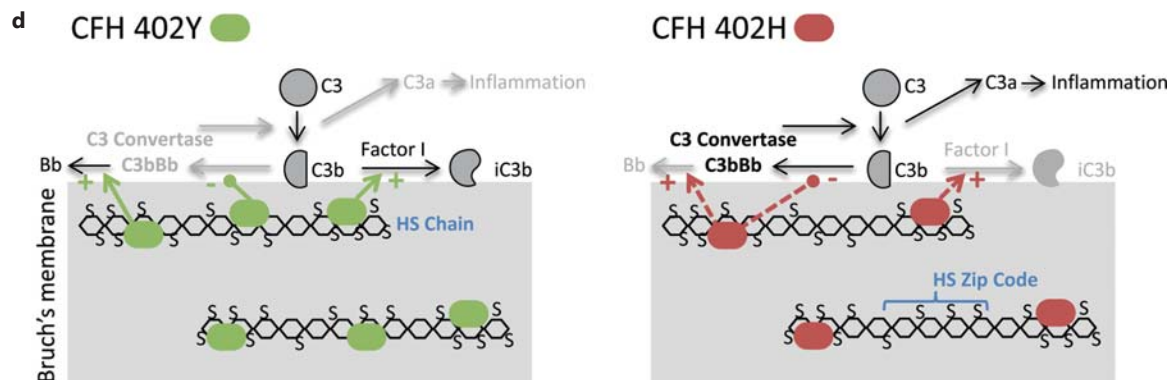
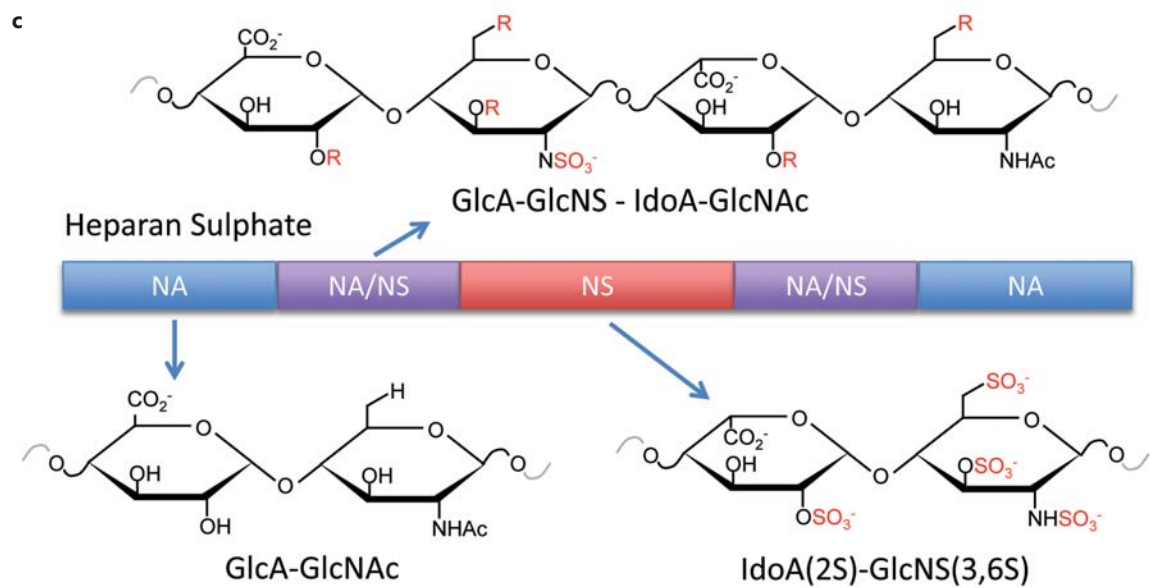
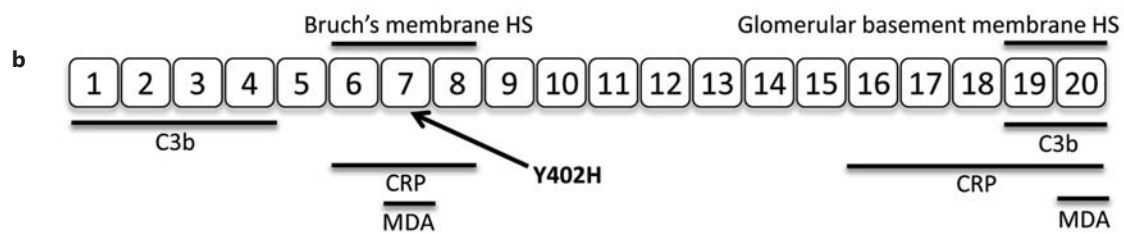
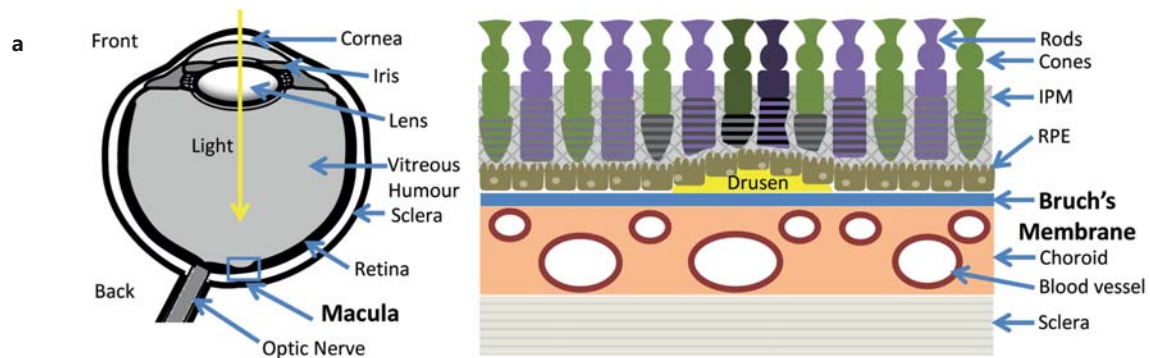
### The Role of CFH in AMD

CFH has a key role in regulating the alternative pathway of complement; this 155-kDa serum glycoprotein is composed of 20 complement control protein (CCP) domains, such that different regions of CFH recognize different ligands (fig. 1b). For example, CFH inhibits the formation of the alternative pathway C3 convertase by competing with factor B binding to C3b [or C3(H<sub>2</sub>O)] via its CCP1–4 region; CFH also promotes the decay of existing C3 convertase by displacing factor Bb. CFH acts as a co-factor for factor I in the inactivation of C3b to iC3b so that

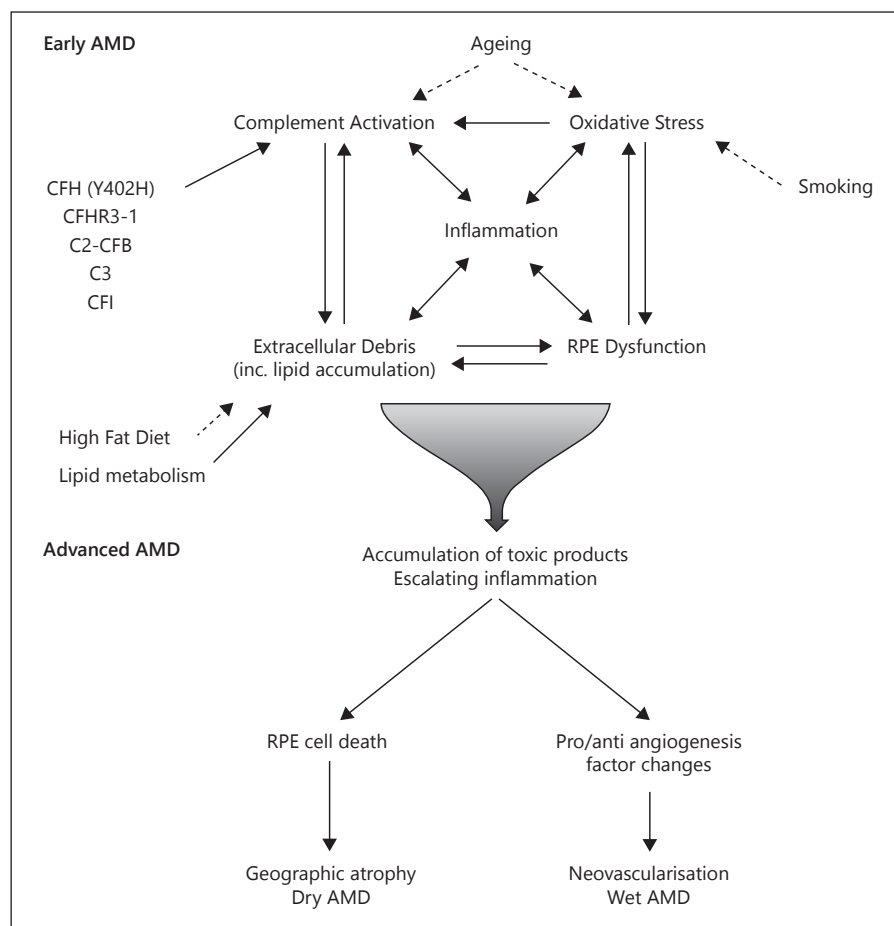
**Fig. 1.** Structures of the eye, CFH and HS; effect of CFH Y402H polymorphism on HS ZIP code recognition and complement regulation in Bruch's membrane. **a** Cross-sections of the eye (left) and the bottom half of the macula (right). IPM = Interphotoreceptor matrix. **b** Modular organization of CFH showing 20 CCP domains, the position of the Y402H polymorphism and regions involved in ligand binding. **c** Domain structure of HS, containing NA, NA/NS and NS domains. Representative structures are shown for the highly sulphated NS domains [i.e. comprising the IdoA(2S)- $\alpha$ 1-4-GlcNS(3,6S) disaccharide], the NA regions (comprising GlcA- $\beta$ 1-4-GlcNAc) and a possible HS tetrasaccharide from the NA/NS region (comprising GlcA- $\beta$ 1-4-GlcNS- $\alpha$ 1-4-IdoA- $\alpha$ 1-4-GlcNAc). R represents variable sites of sulphation, contributing to the huge diversity in HS structure. **d** Model showing possible functional dif-

ference between the 402H (AMD-associated) and 402Y forms of CFH in Bruch's membrane. The 402Y form of CFH (green) can bind to multiple sites on HS chains in Bruch's membrane due to its wide specificity for HS ZIP codes; the 402H variant (red) only binds to highly sulphated motifs within HS, meaning that fewer molecules of this CFH allotype localize to this extracellular matrix. CFH inhibits complement activation by acting as a co-factor for the factor-I-mediated inactivation of C3b to iC3b; it also prevents the formation and accelerates the decay of the C3 convertase C3bBb. Thus, if insufficient CFH is present in Bruch's membrane, as is the case for the 402H variant, there will be increased activation of the complement cascade and the release of pro-inflammatory mediators, such as C3a.

(For figure see next page.)



**Fig. 2.** Overview of the pathogenesis of AMD. Complement dysregulation [7, 10, 37] and oxidative stress [21, 44] have been implicated in causing/amplifying the inflammation central to AMD pathology. These processes are influenced by genetic effects [2–6, 51, 52] (table 1), denoted by grey arrows, and also environmental factors, highlighted with dashed arrows, including smoking [53] and the effect of a high fat diet [54].



there is less available C3b for the formation of the C3 convertase, C3bBb, or the C5 convertases, C3bBb3b and C4b2a3b [13]. The CCP6–8 and CCP19–20 regions are important for binding to, amongst other things, heparan sulphate (HS), malondialdehyde (MDA) and C-reactive protein (CRP) [16].

Given the severity of the risk and the prevalence of the Y402H polymorphism, there have been a number of studies that have investigated the functional effect on the ligand-binding properties of CFH: a coding change occurs in CCP7 (fig. 1b), and many studies have utilized a recombinant protein comprising CCP6–8 with either a histidine or a tyrosine at position 402 [17]. For example, differential binding of the 402H and 402Y allotypes has been observed to CRP, chondroadherin, DNA, fibromodulin, heparin/HS, MDA, necrotic cells, Shiga toxin and *Streptococcus* M protein [18–22]. As described below, the dramatic effect of Y402H on the interaction with the glycosaminoglycan (GAG) HS could provide a biochemical explanation for the role of this polymorphism in AMD [17, 23, 24]; how-

ever, this does not exclude the possibility that its effect on other ligand-binding activities may also play some part.

Our previous work demonstrated that the Y402H coding change alters the specificity for heparin/HS of CFH [17, 23]. By selectively de-sulphating heparin, as a model of HS, it was shown that the CFH 402H (AMD-associated) variant requires a high degree of sulphation for high affinity binding [17]; however, the 402Y allotype has a much broader specificity for HS, meaning that it can interact with a wider range of HS structures in tissues (see below). Consistent with this, high-resolution structural analyses indicated that the residue at position 402 is directly involved in binding to heparin/HS and that the change from a histidine to tyrosine will likely affect the sulphation pattern that can be recognized [23]. In this regard, we found that, while the two CFH variants can bind similarly to the RPE cell layer in human macular tissue sections, the 402H form binds poorly (approximately 2-fold less than 402Y) to Bruch's membrane, where HS plays a major role in mediating these differential interactions [24].

**Table 1.** Summary of pathways and genes associated with AMD

Function	Gene(s)	References
Complement components and regulators	Complement factor H ( <i>CFH</i> )	[2–4]
	Complement factor H related proteins ( <i>CFHR3-1</i> )	[5, 6]
	Complement component 2 ( <i>C2</i> )	[2, 3, 51]
	Complement factor B ( <i>CFB</i> )	[2, 3, 51]
	Complement component 3 ( <i>C3</i> )	[2, 3, 15, 52]
	Complement factor I ( <i>CFI</i> )	[2, 15]
	Complement component 9 ( <i>C9</i> )	[16]
Extracellular matrix components and regulators	Collagen, type VIII, $\alpha 1$ ( <i>COL8A1</i> )	[2]
	TIMP metalloproteinase inhibitor 3 ( <i>TIMP3</i> )	[2, 3]
	Collagen, type X, $\alpha 1$ ( <i>COL10A1</i> )	[2]
Angiogenesis	ADAM metalloproteinase with thrombospondin type 1 motif, 9 ( <i>ADAMTS9</i> )	[2]
	Vascular endothelial growth factor A ( <i>VEGFA</i> )	[2, 3]
	Transforming growth factor, $\beta$ receptor 1 ( <i>TGFBRI</i> )	[2]
Lipid metabolism	Apolipoprotein E ( <i>APOE</i> )	[2]
	Cholesterol ester transfer protein, plasma ( <i>CETP</i> )	[2, 3]
	Lipase, hepatic ( <i>LIPC</i> )	[2, 3]
Uncertain	Coiled-coil domain containing 109B ( <i>CCDC109B</i> )	[2, 3]
	Age-related maculopathy susceptibility 2 ( <i>ARMS2</i> )	[2, 3]
	HtrA serine peptidase 1 ( <i>HTRA1</i> )	[2]
	Filamin A interacting protein 1-like ( <i>FILIP1L</i> )	[2]

### HS: a ZIP Code for CFH

HS is a GAG that is present on the surface of all cells and in the extracellular matrix; this polysaccharide is attached to particular core proteins to form HS proteoglycans, which have important roles in development, cell differentiation, signalling, tissue homeostasis and disease [18, 25–28]. HS is formed of repeating disaccharides comprising a glucuronic acid (GlcA) or iduronic acid (IdoA) linked to N-acetylglucosamine (GlcNAc) and is typically 50–200 disaccharides in length. The basic repeating disaccharide can be modified by sulphation at various positions to produce a large number of sequence permutations, giving rise to immense structural diversity (as is illustrated in fig. 1c). Additional complexity occurs through the formation of domain structures, with highly sulphated N-sulphated (NS) regions interspersed within N-acetylated (NA) regions that have undergone limited modifi-

cation and consist mainly of GlcA-GlcNAc repeats. The NS domains are typically 3–8 disaccharides long and are modified by N- and O-sulphation, with epimerization of most GlcA to IdoA; NA/NS domains flank the NS domains and comprise alternating N-acetylation and N-sulphation with a mixture of GlcA and IdoA and some variable O-sulphation. The number/organization of these different domains and the variability in the length of the HS chain further add to the complexity of HS.

Overall, the large degree of structural diversity in HS and its ubiquity in tissues allows it to play a major role in the regulation of physiological processes [25–28], i.e. via its interaction with many proteins and through the modulation of their functions. Given that the composition/sequence of HS likely differs from one particular tissue microenvironment to another, this gives HS the potential to act as a ZIP code for a particular cellular/tissue location. In this regard, we have recently mapped the location



of HS and its core proteins within the human eye [29, 30], showing that there is a distinct pattern of HS/HS proteoglycan structures within the different layers of the retina, choroid and sclera; this was performed using antibodies that recognize particular HS epitopes. For example, around the blood vessels of the inner retina (not shown in fig. 1a), there is HS with a high level of 6-O-sulphation, while in the inner limiting membrane, there are HS species with both high and low 6-O-sulphation; given that the binding and regulation of angiogenic growth factors (e.g. fibroblast growth factor 2 and vascular endothelial growth factor) requires 6-O-sulphation, local differences in HS structure may play a role in modulating the formation of blood vessels, e.g. preventing outgrowth into the vitreous from the neurosensory retina [29].

This analysis also showed that there is a diverse range of HS structures within Bruch's membrane, including N-, 6-O- and 2-O-sulphated structures, 3-O-sulphated HS chains as well as structures with a low level of 2-O-sulphation [19]. In this regard, we have previously found that the 402H (AMD-associated) variant of CFH requires the presence of HS with 2-O- and/or 6-O-sulphation to support its binding to human Bruch's membrane, whereas 402Y can interact with HS lacking such sulphation [18, 24]. Thus, given the more restricted specificity of the 402H, which requires highly sulphated structures for binding [17, 23], it seems likely that there may be relatively fewer HS sequences that can be recognized by 402H (compared to 402Y) in human Bruch's membrane, at least in the old donor tissues used in these studies [24, 29]. Interestingly, CFH binding sites were only abundant within the RPE, Bruch's membrane and the choroid (e.g. around blood vessels), with low levels throughout the neurosensory retina (e.g. in the interphotoreceptor matrix) [24]. This indicates that the HS ZIP codes used by CFH are restricted to particular regions, despite HS being present throughout the eye.

Interestingly, the RPE, Bruch's membrane and choroid are sites that come into contact with complement components (e.g. from blood) and therefore require tight regulation to prevent unwanted complement activation; i.e. because of the continuous slow tickover of the alternative pathway that left unchecked would lead to the deposition of C3b and the amplification of complement, resulting in inflammation and tissue damage. While cell surfaces can be protected from complement by a number of endogenous cell-bound regulatory factors such as CD35, CD46, CD55 and CD59 [31], extracellular matrices, such as basement membranes (e.g. in kidney glomeruli and Bruch's membrane in the eye), do not possess

these regulators and need to recruit CFH to protect themselves from aberrant complement activation [18, 20]. CFH also plays an important role in self versus non-self recognition by being recruited to sites on host cell surfaces that contain a suitable localization signal, e.g. including HS ZIP codes [24].

### **Tissue-Specific Host Recognition by CFH via HS ZIP Codes**

CFH contains two HS recognition domains, comprising CCP6–8 and CCP19–20 (fig. 1b), with mutations and polymorphisms in these regions being associated with AMD or the kidney disease atypical haemolytic uremic syndrome (aHUS), respectively, but not with both diseases [see ref. 32, and references therein]. Recently, we have investigated the GAG-binding properties of the CCP6–8 and CCP19–20 regions and have found that the former has a higher apparent affinity for heparin [32]. In terms of sulphate specificity, the CCP19–20 region is much more similar to the 402H variant of CCP6–8 (than 402Y), being highly sensitive to the removal of sulphate groups. However, their specificities are different since the removal of 6-O-sulphates, for example, has a larger effect on the binding of CCP19–20 compared to CCP6–8 402H. Thus, the CCP6–8 and CCP19–20 regions might be expected to recognize different HS structures in human tissues. Consistent with this, we have found that it is the CCP6–8 region that makes the major contribution to the binding of CFH to Bruch's membrane, with CCP19–20 playing only a minor role [32]; previously, we have shown that about 70% of the binding sites for CCP6–8 in Bruch's membrane are composed of HS [24]. Conversely, the CCP19–20 region plays the dominant role in the recognition of kidney glomerular basement membrane by CFH, which again is predominantly mediated by HS [32]. Interestingly, in a recent study, the HS ZIP code recognized by CFH in another region of the kidney (renal tubular epithelial cells) was found to be distinct to that of properdin, a positive regulator of the complement system [33]. This illustrates that different HS ZIP codes within the same tissue can play a role in the differential control of the binding of complement regulatory proteins.

As noted above, the two HS-binding domains of CFH have different specificities allowing the recognition of distinct HS ZIP codes and the tissue-specific localization of CFH [32]. Thus, our studies have provided a biochemical explanation for the organ-specific effects of mutations/polymorphisms in the *CFH* gene, where Y402H (in

CCP7) is associated with AMD, whereas those in CCP19–20 are all associated with aHUS. One exception is the rare penetrant R1210C mutation in CCP20 that confers a high risk of developing AMD [34]. While the mutation is thought to reduce heparin binding [35], more importantly, it also results in CFH, forming a covalent complex with serum albumin [36] that would likely have a dramatic effect on the transport of this complement regulator through Bruch's membrane [32].

### The Role of CFH in AMD Pathogenesis

As noted above, we have found that the AMD-associated 402H form of CFH binds relatively poorly to HS in Bruch's membrane compared to 402Y [24]. In vivo, this could lead to a decreased ability of the 402H variant of CFH to localize to Bruch's membrane, resulting in impaired regulation of the alternative pathway of complement and the production of pro-inflammatory mediators (fig. 1d) [18, 20, 24]. This would be expected to result in chronic local inflammation, which is thought to be a key pathogenic feature in AMD (fig. 2) [7, 10, 37]. It has been shown that the CFH Y402H polymorphism is not associated with significantly increased systemic complement activation [38, 39] adding weight to its function being locally mediated. Consistent with this, individuals who are homozygous for *CFH* 402H have been found to have significantly higher levels of complement activation (e.g. membrane attack complex) and elevated markers of inflammation in Bruch's membrane and/or choroid [40, 41]. However, in addition to the effect of Y402H on HS binding, this polymorphism in *CFH* has also been clearly implicated in other processes that could contribute to inflammation and AMD pathology.

In this regard, oxidative stress results in RPE damage/dysfunction and the generation of the pro-inflammatory MDA by lipid peroxidation of membrane phospholipids [21, 42]. The presence of MDA on the surface of RPE cells acts as a binding site for CFH via CCP7 or CCP20 (fig. 1b), inhibiting complement activation and also the MDA-induced expression of IL-8 by monocytes and macrophages [21]. Given that the 402H variant of CFH has reduced the binding affinity for MDA (compared to 402Y), less CFH will be recruited to the 'oxidized' cell, meaning that complement activation and chronic inflammation may occur. Similarly, pentameric CRP binds to damaged/necrotic cells and undergoes conformational changes to monomeric CRP [43], which then acts as a binding site for CFH. The CFH-CRP interaction is also impaired by the Y402H

mutation, which may lead to increased complement activation and inflammation in the macula of individuals carrying the 402H allele [22, 44].

There are currently many unanswered questions regarding the pathogenesis of AMD. In particular, it is far from clear what initiates the disease process or, indeed, the relative importance/timing of the various mechanisms implicated above; however, as noted before, complement dysregulation and inflammation have been identified as having a central role (fig. 2). One important question is why AMD symptoms only manifest over the age of 50 years (even in individuals with a strong genetic predisposition). Our studies have found that the 402Y and 402H variants of CFH have an inherent difference in their ability to bind HS in Bruch's membrane [24]. Here we used old, but non-AMD, donor tissue, raising the possibility that age-related changes in HS ZIP codes, if they occur in the eye as they have been shown to in the aorta [45], could contribute to the disease mechanism [20]. Further work is needed to determine whether AMD pathology results from normal changes in tissue structure with age in combination with genetic and environmental risk factors.

### Animal Models of AMD – A Different Zip Code?

Animal models have great utility in understanding disease pathogenesis and in testing novel therapeutics. In AMD, a large number of rodent models have been developed [for a review, see ref. 46]; however, none of these models recapitulate all aspects of the human disease. Despite structural similarities between murine and human eyes, mice do not have a macula (i.e. a region of the retina with a higher proportion of cones) and do not suffer from AMD.

Mice lacking CFH have been created and, at 2 years of age, these mice demonstrated some loss of photoreceptors and deposition of complement in the retina [47]; however, a thinning of Bruch's membrane was observed rather than a thickening, as is observed in man [11]. This mouse model indicates that the absence of CFH (with no other initiating factor) leads to dysregulation of the innate immune system causing pathology in the retina. To elucidate the role of the 402Y/H variants, this CFH null mouse was modified to express a humanized form of CFH containing the mouse CCP1–5 and CCP9–20 and the human CCP6–8 containing either the 402H or 402Y variants [48]. Here, an early AMD-like phenotype was observed with drusen-like deposits forming; however, this occurred with both the 402H and 402Y forms. The lack

of a difference between the 402H and 402Y phenotypes may be because the HS ZIP code is not conserved between species [49], such that the human CCP6–8 region cannot recruit the chimeric CFH to Bruch's membrane. Furthermore, it should be noted that mouse models of inflammatory disease often poorly mimic the gene expression changes observed in man [50]. Sadly, no fully satisfactory animal model yet exists for AMD, making its use for the evaluation of potential therapeutic interventions problematic.

## Summary

The structural diversity of HS allows it to be utilized as a versatile ZIP code to specify precise tissue locations. Here, we have described how CFH recognizes particular HS structures, allowing it to bind to host tissues and prevent the spontaneous activation of the alternative complement pathway (e.g. in the human eye). Two different regions of CFH have distinct HS-binding properties providing tissue-specific recognition for Bruch's membrane

(CCP6–8) or the glomerular basement membrane (CCP19–20). Importantly, this tissue specificity at the protein level may help explain the genetic basis of AMD and aHUS and provides new insights into the pathogenic mechanisms for these diseases of complement dysregulation. However, further research is needed to more fully understand how the interaction between CFH and HS ZIP codes contributes to the initiation and progression of AMD.

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## Disclosure Statement

The authors have no financial conflicts of interest.

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